EFFECTS OF ACUTE AND CHRONIC ELEVATED PH EXPOSURE ON SURVIVAL OF HATCHERY FRY AND FINGERLINGS OF SELECT SPORT FISH SPECIES

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Nathan E. Pence, B.A.

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Table of Contents

Acknowledgements iv

Table of Contents v

List of Tables vii

List of Figures viii

Abstract ix

INTRODUCTION 1

METHODS 5

- Fry Collection 5
- Designation of Day-0 5
- Acute Test 6
- Chronic Test 7
- Variations from Described Methods for Bluegill 8
- Statistical Analysis 8

RESULTS 10

- Acute Experiments Results 10
- Chronic Experiments Results 10
- Observations 11

DISCUSSION 12

- Florida Largemouth Bass 14
- Smallmouth Bass 15
- Bluegill 16
- Channel Catfish 17
- Stocking Recommendations 17

TABLES AND FIGURES 20

APPENDIX I: DATA 30

APPENDIX II: LC50 values 33

APPENDIX III ANOVA and Tukey HSD Multiple Comparisons 34

LITERATURE CITED. 37

VITA.......... 45

List of Tables

Table 1. Schedule for addition of NaOH in chronic experiments..... 20

Table 2. Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* tolerance to elevated pH based on the first treatment to yield significant mortality and LC50 values. p values determined by Tukey HSD analysis. 21

Table 3. Effects of acclimation time on % survival of Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* from 0 to 60 min acclimation. * denotes significance by Tukey HSD analysis ($p<0.05$). 22

Table 4. Percent survival of Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* after similar pH manipulations during the acute and chronic experiments..... 23

Table 5. Potential increase in survival of smallmouth bass *Micropterus dolomieu* over an aquaculture production season with 60 min of acclimation time vs 0 min acclimation time. 24

Table 6. Recommended stocking improvements to maximize fry survival and production in fish hatcheries..... 25

List of Figures

Figure 1 6-h survivorship of Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* subjected to acute pH manipulation. Vertical bars denote 95% confidence intervals. 26

Figure 2. 2-h survival Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* after exposure to a chronic 2-unit pH increase (8.1-10.1) over varying time intervals. Vertical bars denote 95% confidence intervals. 27

Figure 3. 6-h LC50 values calculated by the Trimmed-Spearmen Karber method for Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus*. Vertical bars denote 95% confidence intervals. 28

Figure 4. Distribution of Florida largemouth bass *Micropterus salmoides floridanus* and smallmouth bass *Micropterus dolomieu* in the continental United States. Distribution maps are based on compilations of Bailey and Hubbs 1949, Kleinsasser et al. 1980, Maceina 1992, and Maceina et al. 1988 for FLB and Becker 1983, Carlander 1975, Coble 1975, Green 1995, MacCrimmon and Robbins 1975, and Robbins and MacCrimmon 1974 for SMB. 29

Abstract

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by

Nathan E. Pence, B.S.
Aquatic Station-Biology, Southwest Texas State University
December 2002

Supervising Professor: Dr. Tom Arsuffi

Contrasting pH levels between indoor spawning raceways and outdoor production ponds are thought to be a factor contributing to low survival of fry and fingerlings at aquaculture facilities. Standard procedure is to move fry and fingerlings from indoor hatching and holding facilities to outdoor grow out ponds. Often outdoor pH levels are higher than the indoor pH levels and fry and fingerlings are then subjected to those fluctuations in pH. Here I experimentally determined the effects of acute (instantaneous) and chronic (w/ acclimation time) pH changes on fry or fingerlings of the Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus*. To test for tolerances to acute pH levels I conducted single factor experiments with 5 pH treatments (9.0, 9.4, 9.7, 10.0, 10.5) and a control (incoming hatchery water supply w/ pH 8.1) and then used survivorship after 6 h as a response variable. ANOVA showed significant ($p \leq 0.05$) mortality for all four species at varying pH levels: smallmouth bass 10.0, bluegill 10.0, Florida largemouth bass 9.7, and channel catfish 9.4. LC50 values were calculated using the Trimmed-Spearmen Karber method: smallmouth bass 10.22, bluegill 9.87, Florida largemouth bass 9.72, and channel catfish 9.39. Tolerances to pH changes were also evaluated by raising the pH from 8.1 to 10.1 over 5 time intervals (0, 15, 30, 45, and 60 min) and then determining survivorship after 2 h. ANOVA showed significant increase in survival only for the smallmouth bass, however smallmouth bass fry, channel catfish fry, and bluegill fingerlings (% increase in survival @ 60min acclimation time: 29.4, 19.2, 20) all three followed a general pattern of higher survivorship with increased time interval of acclimation to a chronic pH increase. Florida largemouth bass (10% increase in survival @ 60min acclimation time) fry exhibited only small increases in survival with increased time allowed for acclimation. These results suggest it is important for hatcheries to adopt culture methods that account for species-specific pH tolerances to maximize survival of fry and fingerlings.

INTRODUCTION

One major component of water quality that plays a significant role in aquaculture yields is pH levels. General guidelines for the acid and alkaline death points of fish have been established as pH levels 4 and 11, respectively (Boyd 1979). Waters with a pH ranging from 6 5-9.0 are most suitable for fish production in aquaculture (Mackenthun 1969, Piper et al. 1982, and Thurston et al. 1981). However, there is no definite pH range within which a fishery is unaffected and outside of which it is devastated, rather there is a gradual deterioration as the pH level rises or falls outside of a species optimal range. Even if fish do not die from prolonged exposure to elevated pH levels, many species often exhibit stress in the form of reduced growth, reduced reproduction, or impairment of other bodily processes (Piper et al. 1982). Shifts in pH levels typically irritate and stress the ionoregulatory process of the fish (Ingersoll et al. 1990) and result in compensatory responses such as fish covered with mucous, swollen gills, and bursting capillaries (Bulkley 1975, Calabrese 1969, Daye and Garside 1976, Daye and Garside 1980, Ingersoll et al. 1990, Trama 1954). Stress and resulting compensatory responses are associated with pH levels as low as 9.0 in some species (Daye and Garside 1976, Serafy and Harrell 1993). A high pH also causes stress or mortality because the amount of toxic un-ionized ammonia in the water column is positively related to the pH of the

water (Bergerhouse 1993, Boyd and Tucker 1992, Haywood 1983, Thurston et al. 1981, Witschi and Ziebel 1979). Generally, an increase of one pH unit in the water results in a shift from the non-toxic ammonium ion to the toxic un-ionized ammonia fraction with a 10-fold increase in the percentage of toxic un-ionized NH_3 (Haywood 1983, Thurston et al. 1981). Ammonia in the water column leads to chronic gill necrosis (Robinette 1976, Tomasso et. al. 1980) and will eventually become lethal.

Warm water aquaculture facilities are specifically concerned with pH because methods to maximize production of fry and fingerlings (Bergerhouse 1993) or the time of year a species is reared often contribute to elevated pH levels in rearing ponds. During spring production for species whose fry and fingerlings are zooplanktivorous, the most widely practiced pond management tool for promoting an adequate forage base for fingerling fish in outdoor ponds is fertilization (Barkoh 1996). Elevated pH levels result from fertilizers (nitrogen, phosphorus, and carbon), which stimulate phytoplankton growth, causing a high rate of photosynthesis in the pond (Bergerhouse 1993, Ludwig et al. 1998, Morris and Mischke 1999, Piper et al. 1982). The phytoplankton and the organic fertilizers provide a food base for larger zooplankton, which is the main food source of the growing fry and fingerlings (Ludwig et al. 1998, Morris and Mischke 1999). In contrast, fry of species reared during summer production are fed with an artificial diet of commercially prepared pellets. Since the growing fry do not depend on plankton as a food source, the ponds are not fertilized. However, even these ponds during summer have high pH levels because of high photosynthesis rates caused by continuous hot sunny days

Rapid changes in the pH of the water over a short duration of time can stress fry

and cause mortality (Bergerhouse 1992, Piper et al. 1982, Witschi and Ziebel 1979). A rapid change in pH is commonly encountered in aquaculture when moving fish from one water source to another. Fry that are hatched indoors and reared outdoors must be moved from indoor hatching facilities with fairly stable and acceptable pH levels to outdoor rearing ponds that often exhibit drastic shifts in pH levels. Elevated pH in the ponds at the time of fry stocking from indoor hatching facilities to outdoor rearing ponds may be one cause of low survival for various species in many hatcheries (Bergerhouse 1992, 1993). To help fish cope with the change in pH associated with transfer, an established culture technique is to temper fry before they are transferred from one water source to another with different water quality parameters (Piper et al. 1982). Tempering is the process of gradually adding water from the new destination to the water that fry are moved from to allow fry to slowly acclimate to new water quality conditions.

Numerous studies show that various species have dramatic reactions to elevated pH levels and that these reactions vary among different species (Barkoh 1996, Bergerhouse 1992, 1993, Calabrese 1969, Daye and Garside 1975, Daye and Garside 1976, Eipper 1975, Jordan and Lyod 1964, Lyon and Fisher 1998, Stiemke and Eckenfelder 1947, Tomasso et al.1980, Trama 1954, Wiebe 1931). Therefore aquaculture facilities, which raise species with undetermined pH tolerances, should be concerned with understanding how pH affects survival of species cultured by their facilities.

The goal of this research was to determine if pH is a factor affecting fry and fingerling survival of four sportfish species reared by A E Wood Fish Hatchery (AEW), Texas Parks and Wildlife, San Marcos, TX. Specifically, to determine the effect of pH

on survival of Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus*. To do this, I determined pH tolerance of fry/fingerlings by experimentally evaluating survival after: 1) acute exposure to 5 pH concentrations: 9.0, 9.4, 9.7, 10.0, 10.5, and 2) after graduated exposure to a chronic 2 unit pH increase over 5 time intervals: 0, 15, 30, 45, 60 min. Acute experiments were to establish at what pH mortality starts and at what pH levels it becomes significant. Chronic experiments were to determine if increased acclimation time during stocking would increase survival of fry and fingerlings.

METHODS

Fry Collection

Florida largemouth bass and channel catfish eggs were collected from indoor raceways at AEW. Florida largemouth bass eggs were collected and allowed to hatch naturally in hatching troughs. The Florida largemouth bass eggs used for research were collected and hatched in late March and early April. Channel catfish eggs were hatched in McDonalds jars. Eggs used for the channel catfish research were collected in mid May.

Smallmouth bass and bluegill were spawned in ponds at AEW. Smallmouth bass eggs were collected and moved indoors to hatch in the incubation facilities. Smallmouth bass eggs were collected and used for research in mid April. However bluegill eggs were allowed to hatch naturally in the pond and then fingerlings were collected. Bluegill used were collected in late July and early August.

Designation of Day-0

To ensure that all fry were at the same stage of development for use in experiments, I used the following procedure to define d-0. Because water temperature increases over the production season, it is necessary to define d-0 by development of the young rather than by the actual day the eggs are laid or hatch. The number of h required

for the eggs to hatch decreases as the water temperature increases. For example, at 10, 18 and 28 degrees, it takes 317, 55 and 49 h respectively for eggs to incubate and hatch (Heridinger 1975). At hatching the fry are small, typically ranging from 3 to 5 mm in total length, depending on the species (Coble 1975, Heridinger 1975). At this time they have no mouth to feed and obtain all their nourishment from the yolk sac. When first hatched, the larval fry are unable to assume an upright position or swim; they must lie on their sides in the nest for a period of a 6 h to d-2. This time is again dependent on water temperature (Coble 1975). The larvae are only able to right themselves and rise from the nest after the swim bladder begins to inflate and the yolk sac is completely absorbed. For my study, fry were allowed to develop and absorb their yolk sac. Once this was complete, fry swam up in the water column, and were collected and used for the acute and chronic tests that day; these fry were designated d-0.

Bluegill spawning methods did not allow for the collection of fry directly from the ponds, and they are not spawned indoors. For this reason, bluegill fingerlings (and not fry) were collected from the ponds at the time of pond harvest and used in pH experiments.

Acute Test

A research room was prepared at AEW and a bench (4 m long and 1.2 m tall) was constructed to accommodate the 18 McDonald hatching jars and other equipment that was used. The jars were filled with 1000 ml of water from the incoming water supply that supplies the hatching troughs, tanks, hatching rack, and raceways. A low-pressure airline was supplied to each jar, with regulator valves to control the amount of air to each jar. The air was restricted to a level to prevent physically stressing the fry with

turbulence, which could over-power and cause the fry to fight and strain but, sufficient enough to ensure that oxygen is not depleted and to keep the water in the jar uniform. Three jars were designated as controls and remained at the initial pH of the fry's water source throughout the duration of the experiment. The pH of the incoming water at AEW was 8.1 and was used as the control for all species. The other 15 jars were used in replicates of 3 for each of 5 treatments. The treatment pH levels were 9.0, 9.4, 9.7, 10.0 and 10.5. Past research indicates that mortalities associated with NaOH is most reflective of pH change in the water and not toxicity of the chemical itself (Bergerhouse 1992 and 1993, Calabrese 1969, Stiemke and Eckenfelder 1947, Trama 1954). Therefore NaOH (0.1M) was used to titrate the pH to the desired treatment level. The amount of NaOH to reach each individual treatment pH was predetermined prior to the tests. A Miluakee Smart pH meter was used to establish and monitor pH levels. After all 18 jars were set and pH levels stabilized, 50 fry were transferred from their source into each of the 18 jars. Fry were transferred with a 5 ml Oxford Macro-set pipettor to reduce handling stress. Survival was determined at the end of 6 h by counting the number of mortalities in each jar. If the fry did not respond by moving when touched with a probe, they were scored as a mortality. The pH was monitored every 1 h to ensure that the pH of each treatment did not deviate. The acute experiment was repeated twice for a total of six replicates for each of the treatments.

Chronic Test

For the chronic study, the setup of the jars and airlines was the same as for the acute study. The difference was that 50 fry were added prior to manipulation of the pH in the jars with NaOH Three jars were used as the controls and remained unchanged

through the duration of the experiment. The other 15 jars were divided into replicates of 3 among 5 treatments. 0, 15, 30, 45 and 60 min. Each treatment represents the amount of time taken to titrate each of the treatments 2 pH units (from 8.1-10.1). The amount of NaOH required to titrate the pH level by 2 pH units was determined and applied every 5 min over the treatment time interval (Table 1). After the pH reached the treatment pH, the fry were monitored for 2 h. A Miluakee Smart pH Meter was used to establish and monitor pH levels. After 2 h, survivorship was determined as in the acute test.

Variations from Described Methods for Bluegill

Bluegill was the only species whose methods varied from those previously described. Due to difficulties in collecting bluegill fry, fingerlings (a mean length of 16.8 mm and a range 11-28 mm) were collected at the time of pond harvest and used for the study instead of fry. Also, during the acute study the bluegill fingerlings were observed surviving past 4 h of observation and it was not until 5-6 h that the majority of the observed mortalities occurred. For this reason the chronic experiment was observed for 6 h instead of 2 h of observation used for the other fish species. Also only 3 replicates were performed for the bluegill acute experiments instead of 6.

Statistical Analysis

Data from the 3 or 6 replicates (depending on the species and experiment) for each individual treatment of a species were combined to form a mean percent survival for each treatment pH level. One way analysis of variance (ANOVA) was used to determine if significant mortality occurred ($p \leq 0.05$) for each experiment and Systat 9 statistical software was used to perform Tukey HSD multiple comparisons analysis to determine at which treatment pH significant mortality occurred ($p \leq 0.05$). A MSE was calculated for

each species and 95% confidence intervals determined. LC50 values were calculated using the Trimmed Spearman-Karber method (Hamilton et al. 1977, Montana State University 1992).

RESULTS

Acute Experiments Results

All four sportfish species exhibited increased mortality to acute incremental pH concentrations and all had near 100% mortality at a pH of 10.5 (Figure 1). ANOVA showed significant mortality ($p \leq 0.05$) for all four species in the acute experiments, but the patterns of tolerance varied among species (Figure 1). The smallmouth bass showed the highest overall tolerance to elevated pH levels with an overall survival of 92.5% at a pH of 10.0 after 6 h (Figure 1) and an LC50 of 10.24 that was 0.5 pH units greater than the other 3 species (Figure 3). Channel catfish had the lowest overall tolerance to pH out of the four species tested with only 12% survival at a pH level of 9.7 after 6 h (Figure 1) and an LC50 value of 9.41 (Figure 3).

Chronic Experiments Results

Increased acclimation time resulted in increased survivorship for all four sportfish species but ANOVA showed significant increases in survival only for smallmouth bass ($p=.01$). Magnitude and the degree of increased survival varied among species (Figure 2). All four species exhibited increased survival from 10% to 29.4% at 60 min acclimation time and the mean increase in survival for all four species combined was 20% at 60 min acclimation time (Table 3).

Observations

Just preceding death, the fry swam around violently near the surface, and were disoriented, bumping into the sides of the jars. This behavior is also typical of fish in a natural environment just before death and is documented in other similar pH research (Calabrese 1969, Trama 1954). After death the fry and fingerlings turned a milky opaque color and swelled. The mortalities were also characterized by open mouths and swollen gills. This made scoring mortalities in both the acute and chronic experiments accurate and simple. Most observed mortalities in the jars occurred in the first 2 h of observation. Bluegill fingerlings were the only species that survived past the first 2 h and exhibited most mortalities after 4 h.

DISCUSSION

Results from my acute and chronic experiments suggest that age affects the length of time that a given pH level can be tolerated before mortality occurs. In both the acute and chronic experiments, mortalities were generally observed in the first 2 h of observation for Florida largemouth bass, smallmouth bass, and channel catfish fry. However, no mortalities were observed in the bluegill fingerling jars at the same treatment pH until after the first 3 h and most mortalities were not observed until after 5 h of observation. Fish age affects toxicity of pH levels (Bergerhouse 1992 and 1993, Doudoroff and Katz 1950, Hopkins 1928). In my research older fish had increased tolerance to pH; however, some studies show variable effects where older fish can have greater or reduced tolerance to pH. Bergerhouse (1993) found that d-4 hybrid striped bass fry were less tolerant to elevated pH levels than d-2 larvae, because d-4 fry used sensitive undeveloped gills for respiration whereas d-2 larvae used more resistant cutaneous respiration This same pattern of older fry being more sensitive than younger fry also occurs in channel catfish, walleye, and northern pike (Bergerhouse 1992). The bluegill fingerlings that lived longer than fry of other species in my research were d-30 and had completely developed gills Hopkins (1928) found 4-6 month trout died at a pH range of 7.9-8.9, while fry did not survive beyond a pH of 8.0.

In an aquaculture pond, elevated pH levels are usually diel and temporary in nature and therefore create an advantage for fry or fingerlings that can tolerate elevated pH levels for longer periods of time. Hot sunny days cause high pH levels that spike for a short period of time in the afternoon, followed by falling pH levels at sunset and during the night. Since older fingerlings can survive the same pH levels as younger fish for longer durations of time, older fingerlings would be less vulnerable to diel elevation in pH levels. Fish hatcheries should therefore be particularly concerned with ponds that contain the fry and fingerlings of the youngest age and to ponds that exhibit elevated pH levels that last for longer durations of time.

Within a species, every individual population or ecotype has a unique genotype evolved by selection for a specific local environment (Begon et al. 1990, Mayr 1954, Mayr 1963, Mayr 1976) Such ecotypes have tolerances to pH levels determined by the conditions and parameters of their specific native range and environment. Fields et al. (1987) predicted that stocks of pure northern largemouth bass from Texas, Illinois, and Wisconsin would not react the same to environmental stressors and parameters because of the differences in environment of their native habitats Maceina and Murphy (1992) and Philipp and Whitt (1991) debate the exact cause of the higher mortality of Florida largemouth bass than northern largemouth bass in central Illinois ponds, but both attribute the higher mortality to preset life history characteristics determined by the geographic range of the Florida largemouth bass. Hart (1952) found that the common shiner *Notropis cornutus*, the mosquito fish *Gambusia affinis*, and the northern largemouth bass *Micropterus salmoides* all have distinct populations that exhibit differences in physiological thermal tolerances determined by geographic variations

Zimmerman and Richmond (1981) found that after 40 generations minnows adapted and changed genetically to varying thermal regimes.

While ecotypic variation applies to within a species, variation among different species may also be due to geographic variation. Thoday (1953) distinguished three classes of species with respect to selective forces in their native environments: 1) Those that live in a relatively uniform and stable environment and will therefore not be exposed to selection in favor of genetic or phenotypic flexibility; such species will be selected primarily for stability and adaptation to the uniform environment, 2) Those that live in a fluctuating environment and will therefore be strongly selected for genetic and phenotypic flexibility, especially if the generation time is long, and 3) Those that live in an highly unstable environment and will therefore be strongly selected for genetic flexibility. Depending on the native range and degree of water quality fluctuation typically encountered, a species may have the ability to handle large ranges or small ranges of varying pH levels. To assess this, pH tolerances were compared to geographical native ranges.

Florida Largemouth Bass

The Florida largemouth bass in my research had next to the lowest tolerance to pH and had the least response to increased acclimation time of the 4 species tested. This sensitivity to pH is likely a result of the Florida largemouth bass being an isolated subspecies and ecotype originally confined to a small native range in peninsular Florida (Figure 4a). This area of Florida is where largemouth bass for the TX Florida largemouth bass stocking program originated and is characterized by a more constant climate, a longer growing season, and more stable pH levels than the lakes Florida largemouth bass

have recently been introduced into. Historically the highest pH level recorded by The United States Geological Survey Water Resources (http://www.usgs.gov/) for the lakes and rivers Florida largemouth bass were collected from was 8.8. pH tolerances for the Florida largemouth bass in my research were consistent with these historical pH levels the Florida largemouth bass typically experience within their native range. At a pH level of 9.0-9.4 (uncommon in Florida largemouth bass native habitat) the Florida largemouth bass in my research exhibited 13-21% mortality (Figure 1), a range that to a hatchery manager is approaching the upper limit of acceptable losses, above which economically significant mortalities will occur. Adaptations to mild and stable pH levels in Florida by the Florida largemouth bass may contribute to increased fitness of the Florida largemouth bass in Florida, however they may cause a decline in fitness outside of peninsular Florida where pH levels may be more variable and extreme.

Smallmouth Bass

The smallmouth bass historically has a very large native range and has been introduced successfully across the continental U.S. (Figure 4b) and consequently was exposed to a large range of water quality conditions. This suggests that the smallmouth bass is likely to have a broad tolerance to water quality variation. My results indicate that the smallmouth bass indeed has a high tolerance to elevated pH levels, with survival in the acute experiment above 90% until above a pH of 10.0. Past research indicates the same trend of tolerance above 10.0 to pH levels by smallmouth bass (Calabrese 1969, Wiebe 1931). My research identified the range from 10.0-10.5 as critical for the smallmouth bass (Table 4). Such high tolerance to elevated pH levels is indicative of the large native range and selection for genotypic and phenotypic flexibility.

My results also indicate that smallmouth bass survival increased by 29.4% with an acclimation time of 60 min (Figure 2), the largest and only statistically significant ($p=0.013$) increase in survival associated with increased acclimation time of the four species tested (Table 3). This suggests that tempering could increase smallmouth bass fry survival at the time of stocking. When evaluated over the entire length of a smallmouth bass production season for an individual hatchery, total increase of initial survival after stocking into rearing ponds with tempering increases overall survival of fry by 1.2 million fry (Table 5). Tempering is effective (as in the case of the smallmouth bass) and one factor in increasing survival and maximizing production that hatchery managers can control and manipulate (Table 6).

Bluegill

Bluegill sunfish are native to most of the United States and inhabit waters that represent both extremes of the pH scale. Accordingly, the bluegill fingerlings in my research exhibited significant mortality ($p=0.01$) at a pH of 10.0 (Figure 1) and had an LC50 of 9.87 (Figure 3), which is consistent with past research. Using distilled water and NaOH to raise the pH, Stiemke and Eckenfelder (1947) reported an average "death point" for bluegill was a pH of 10.55 and Trama (1954) found the upper pH limit of bluegill fingerlings was 10.35. In my research the bluegill fingerlings lived for longer durations of time at the same pH as did the fry of the other 3 species. Even though their tolerance to actual pH levels was not the highest, my results showed overall survival could be the greatest of the four species tested if the duration of the pH spike was over 2 h and not over 4-5 h.

Channel Catfish

Channel catfish like the smallmouth bass and bluegill have a large and variable native range, but its pH tolerance is more reflective of the specific niche it occupies in its native range. Channel catfish usually inhabit deeper waters that are characterized by lower pH levels and fewer fluctuations. This resulted in the channel catfish in my research exhibiting the lowest tolerance to pH in this study. Water quality is a major factor that affects channel catfish yields in aquaculture and is the major factor limiting growth of channel catfish cultured in high-density tanks (Andrews et al. 1971). Tukey HSD analysis showed statistically significant mortality at a pH of 9.4 ($p=.01$), and is consistent with previous pH tolerances for channel catfish determined by Ludwig et al. (1998). ANOVA for the chronic results yielded no significance ($p=0.11$) with 3 replicates. However, if more replicates were used the increase of 19% survival associated with 60 min acclimation time might progress from economically significant for hatchery managers to statistically significant.

Stocking Recommendations

Although aquaculture ponds are generally designed to be homogenous to provide uniform growing conditions to maximize production, they can be manipulated to simulate natural spatial heterogeneity in water quality conditions. In laboratory and field studies areas of refugia have been utilized by fish to avoid water of unsuitable pH levels (Bishai 1962, Breck et al.1988, Doudoroff and Katz 1950, Hill et al.1981, Jones 1948, Muniz and Leivestad 1980, Serafy and Harrell 1993). Water inlet valves to the aquaculture ponds could be used to provide a region of temporary refugia (Table 6). Incoming water to the pond has a pH of 8.1, the same as indoor water where the fry are stocked from. When

water is added to the pond it does not mix instantly and if turned on 1-2 d before the stocking of fry, it would create a temporary refugia around the valve.

Because high and rapid changes in pH levels are associated with fry mortality, stocking methods should incorporate practices that promote the stocking of fry and fingerlings when pond pH levels are lowest. Time of day is the easiest way to promote stocking at lower pH levels. Since pH levels typically increase as the day progresses and gets hotter, fry should be stocked just before or after daylight (Table 6). This practice would also allow the fry to gradually acclimate to higher pH levels over the course of the day as the pond pH level increases. It would also allow the fry time to locate either natural or artificial areas of refugia as pH levels increase through the day.

Even though the chronic data yielded only one statistically significant increase in survival, tempering did raise the survival for all four species tested (Figure 2). There were only 3 replicates per treatment for the chronic study and increased replication could show survival was statistically significant for the other species as well. Even though not statistically significant, my results suggest over the course of a production season the number of fry saved by tempering would be economically significant (Table 5) and suggests tempering methods should be established for all species (Table 6). I suggest that all fry be tempered and acclimated when stocking because tempering allows not only acclimation for pH differences but also for other variables that may exist in aquaculture ponds.

Finally, a critical pH threshold level should be established for each fish species. These threshold levels should be set specifically for each facility, according to water chemistry. Also these levels should be set specifically for each species being reared,

according to the species tolerance and age of fry or fingerlings involved. These threshold levels should represent the maximum biological and economical loss acceptable to the hatchery manager. When these levels are surpassed, fry are not stocked into the ponds but are held until the pH again reaches acceptable levels (Table 6).

TABLES AND FIGURES

Table 1. Schedule for addition of NaOH in chronic experiments

	Treatment					
	control	0 min	15 min	30 min	45 min	60 min
Total # ml NaOH added	0	32	32	33	34	36
# ml NaOH added/5 min			10.5	5.5	3.8	3
# of times NaOH added		1	3	6	9	12

Table 2. Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* tolerance to elevated pH based on the first treatment to yield significant mortality and LC50 values. p values determined by Tukey HSD analysis.

Species	pH	% survival	$p_{(q\alpha\ 05,30,6)}$	LC50
1.SMB	10.0	94	0.003	10.2
2.BLG	10.0	40	0.002	9.9
3.FLB	9.7	64	0.031	9.7
4.CCF	9.4	46	0.00001	9.4

Table 3. Effects of acclimation time on % survival of Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* from 0 to 60 min acclimation. * denotes significance by Tukey HSD analysis ($p<0.05$).

	Species			
	FLB	SMB	CCF	BLG
% survival at 0 min acclimation time	37.4	24.0	8.8	6.8
% survival at 60 min acclimation time	47.4	53.4	28.0	26.8
% increase in survival	10.0%	*29.4%	19.2%	20.0%

Table 4. Percent survival of Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* after similar pH manipulations during the acute and chronic experiments.

Species	Acute 8.1→ 10.0	Chronic 8.1 → 10.1	Acute 8.1 → 10.5
FLB	32	37	0
SMB	92	24	2
CCF	3	9	0
BLG	40	7	0

Table 5. Potential increase in survival of smallmouth bass *Micropterus dolomieu* over an aquaculture production season with 60 min of acclimation time vs 0 min acclimation time.

0 min acclimation time	60 min acclimation time
24% survival	53% survival
avg stocking of 200,000 fry/ pond	avg stocking of 200,000 fry/ pond
20 ponds stocked in a production year	20 ponds stocked in a production year
200,000 x 24 x 20	200,000 x 53 x 20
1 million fry survive initial stocking	2 2 million fry survive initial stocking

Table 6 Recommended stocking improvements to maximize fry survival and production in fish hatcheries

Suggested stocking practice	Rationale
1 Stock fry into area of pond that has been flushed with fresh water as a refugia.	Fry have the ability to detect chemical gradients and choose the more favorable The fresh water has a lower pH than the pond water and can act as small temporary refugia at the time of stocking.
2.Stock fry at daylight	pH levels in aquaculture are the lowest in the early morning.
3.Temper all fry for at least 30 min.	Tempering fry increases initial survival and may account for pH and other differences in water quality
4.Cut-off threshold	Establish pH level where the known fry losses are not acceptable. Each facility should set cut-off pH levels according to species raised and specific water quality.

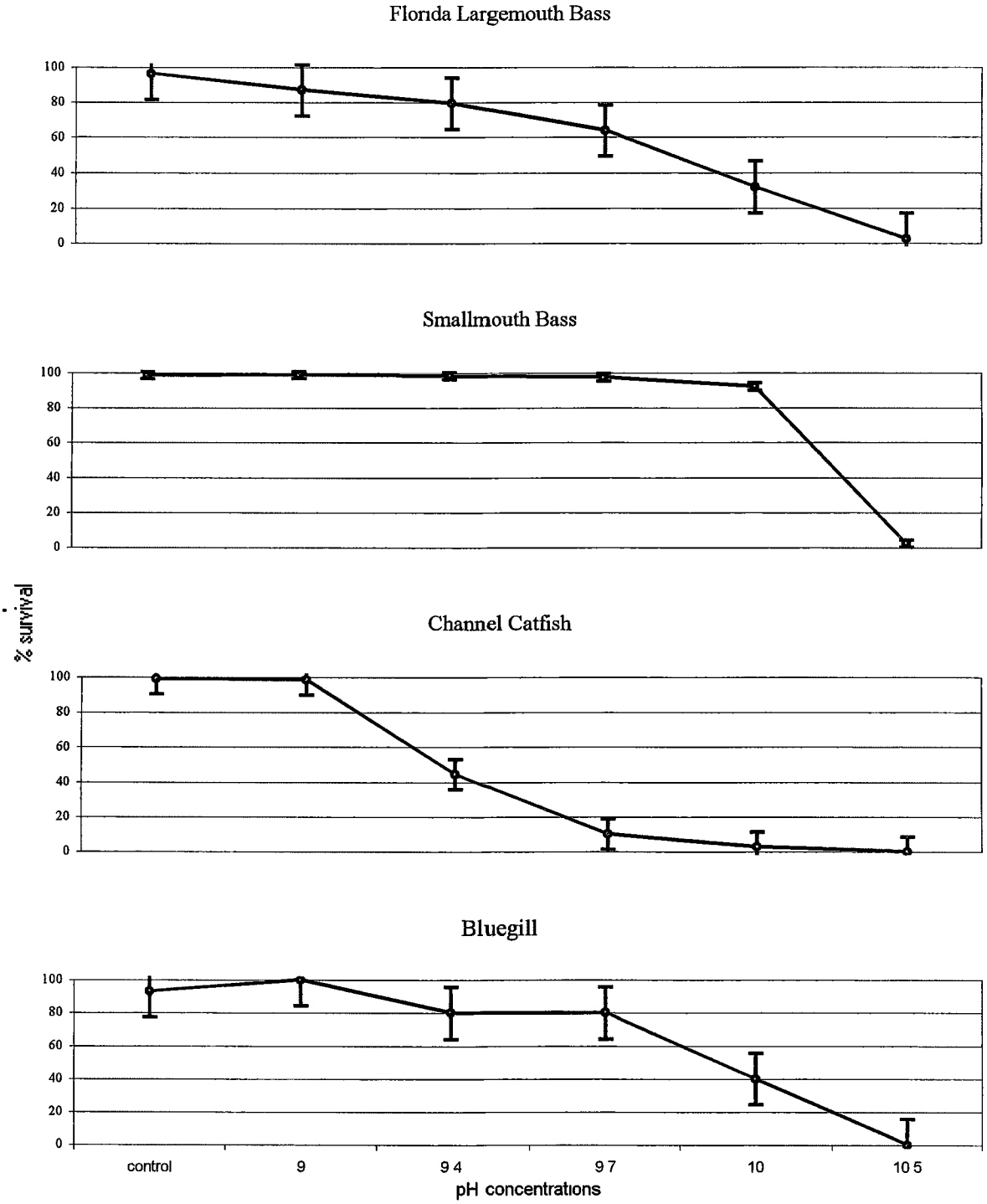

Figure 1. 6-h survivorship of Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* subjected to acute pH manipulation. Vertical bars denote 95% confidence intervals.

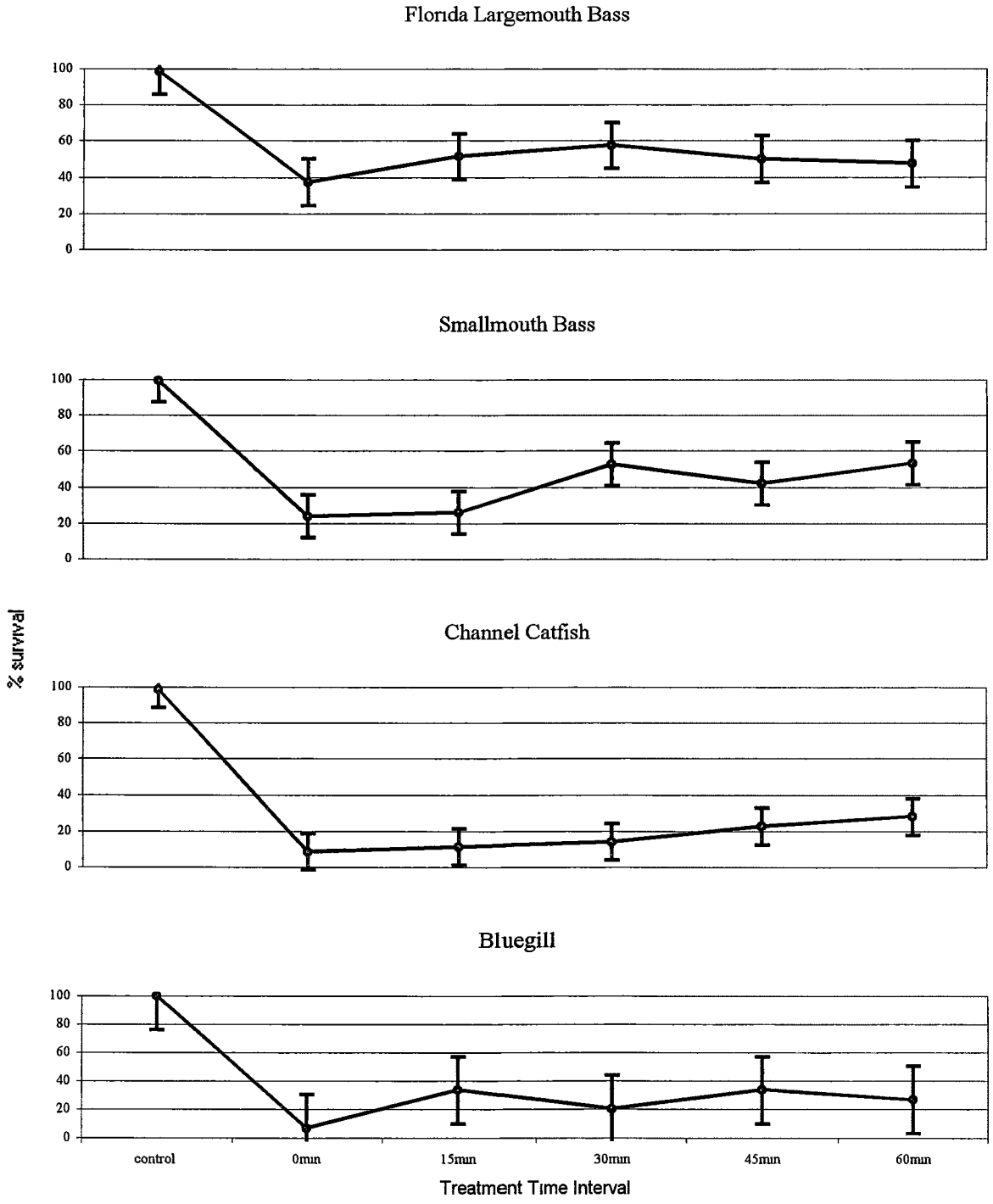

Figure 2. 2-h survival Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus* after exposure to a chronic 2-unit pH increase (8.1-10.1) over five time intervals. Vertical bars denote 95% confidence intervals.

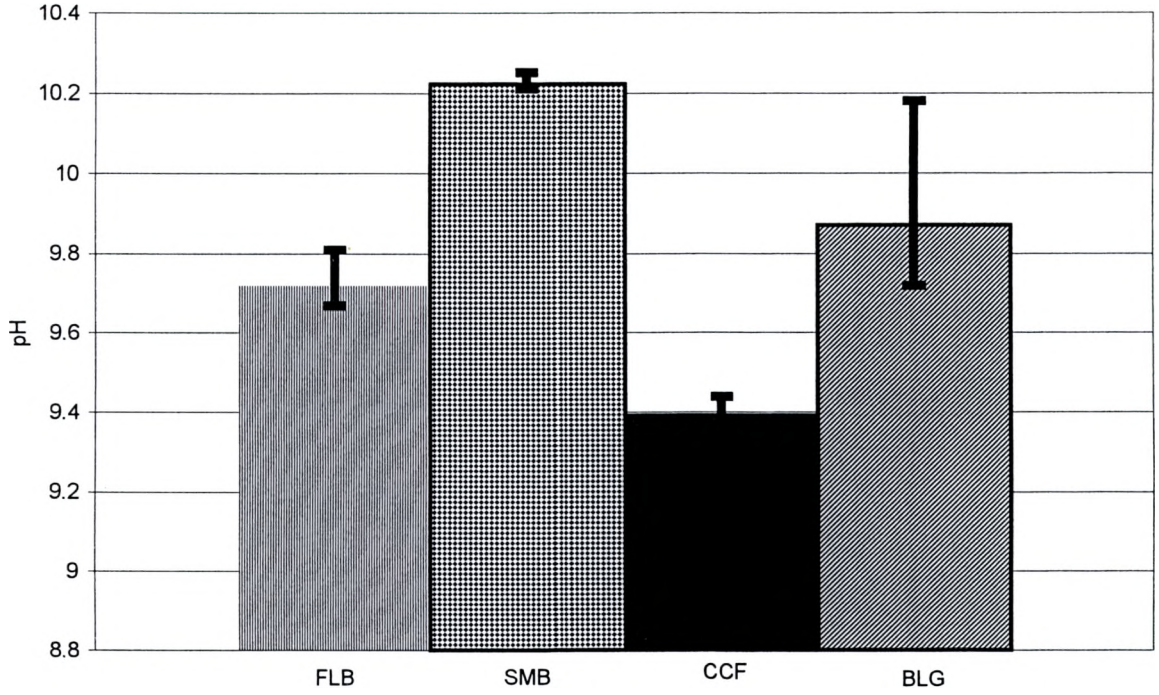

Figure 3. 6-h LC50 values calculated by the Trimmed-Spearmen Karber method for Florida largemouth bass *Micropterus salmoides floridanus*, smallmouth bass *Micropterus dolomieu*, channel catfish *Ictalurus punctatus*, and bluegill *Lepomis macrochirus*. Vertical bars denote 95% confidence intervals.

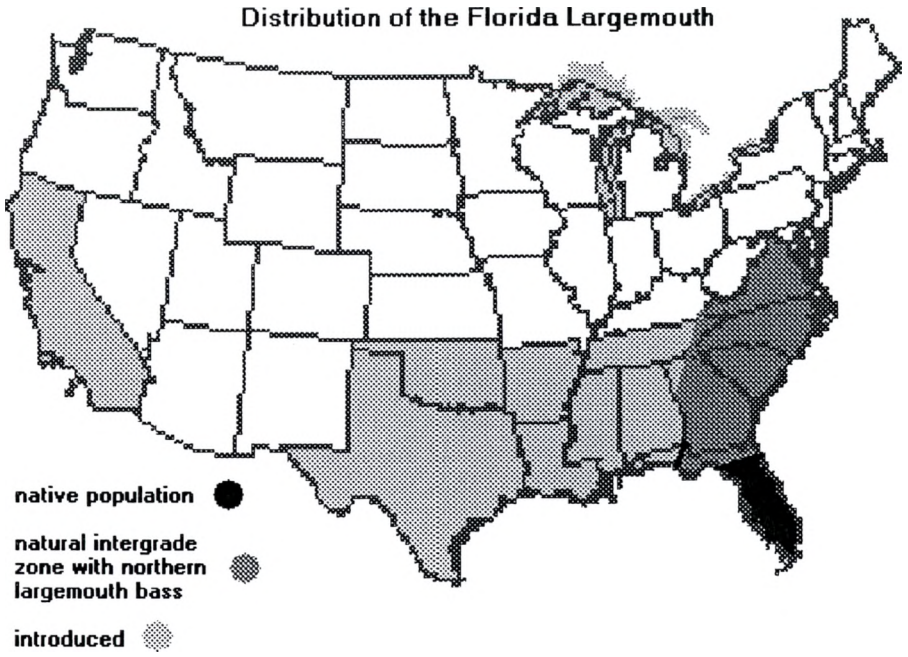

Figure 4a. Distribution of Florida largemouth bass *Micropterus salmoides floridanus* in the continental United States. Distribution map is based on compilations of Bailey and Hubbs 1949, Kleinsasser et al. 1980, Maceina 1992, and Maceina et al. 1988.

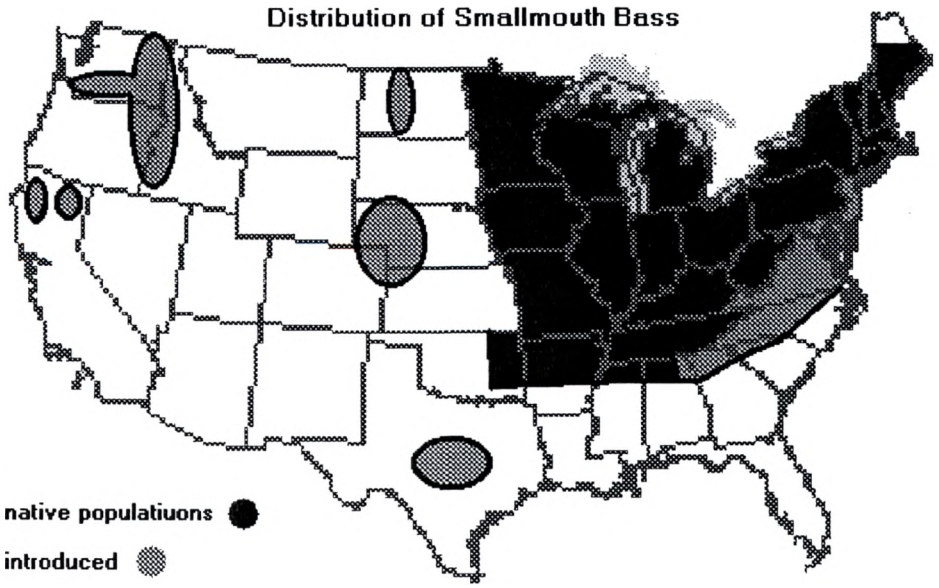

Figure 4b. Distribution of the smallmouth bass *Micropterus dolomieu* in the continental United States. Distribution map isbased on compilations of Becker 1983, Carlander 1975, Coble 1975, Green 1995, MacCrimmon and Robbins 1975, and Robbins and MacCrimmon 1974.

APPENDIX I: DATA

FLB ACUTE 1

	Treatment	Morts
Jar 1	Control	2
Jar 2	Control	2
Jar 3	Control	3
Jar 4	9.00	6
Jar 5	9.00	4
Jar 6	9.00	12
Jar 7	9.40	4
Jar 8	9.40	7
Jar 9	9 40	7
Jar 10	9.70	1
Jar 11	9.70	8
Jar 12	9.70	6
Jar 13	10.00	15
Jar 14	10 00	22
Jar 15	10 00	28
Jar 16	10.50	44
Jar 17	10.50	50
Jar 18	10.50	48

FLB ACUTE 2

	Treatment	Morts
Jar 1	Control	2
Jar 2	Control	2
Jar 3	Control	0
Jar 4	9.00	9
Jar 5	9.00	4
Jar 6	9 00	4
Jar 7	9.40	16
Jar 8	9.40	13
Jar 9	9.40	15
Jar 10	9.70	29
Jar 11	9.70	31
Jar 12	9.70	35
Jar 13	10 00	47
Jar 14	10 00	48
Jar 15	10.00	44
Jar 16	10.50	50
Jar 17	10.50	50
Jar 18	10.50	50

SMB ACUTE 1

	Treatment	Morts
Jar 1	Control	0
Jar 2	Control	0
Jar 3	Control	0
Jar 4	9.00	1
Jar 5	9.00	0
Jar 6	9.00	1
Jar 7	9.40	1
Jar 8	9 40	0
Jar 9	9.40	0
Jar 10	9 70	1
Jar 11	9.70	1
Jar 12	9.70	0
Jar 13	10 00	2
Jar 14	10.00	4
Jar 15	10.00	3
Jar 16	10 50	46
Jar 17	10 50	49
Jar 18	10.50	50

SMB ACUTE 2

	Treatment	Morts
Jar 1	Control	0
Jar 2	Control	2
Jar 3	Control	2
Jar 4	9.00	0
Jar 5	9.00	0
Jar 6	9.00	1
Jar 7	9.40	0
Jar 8	9.40	3
Jar 9	9 40	1
Jar 10	9 70	2
Jar 11	9.70	3
Jar 12	9 70	0
Jar 13	10 00	5
Jar 14	10 00	2
Jar 15	10 00	7
Jar 16	10.50	48
Jar 17	10 50	50
Jar 18	10 50	50

CCF ACUTE 1

	Treatment	Morts
Jar 1	Control	0
Jar 2	Control	0
Jar 3	Control	1
Jar 4	9 00	1
Jar 5	9.00	0
Jar 6	9 00	0
Jar 7	9.40	21
Jar 8	9.40	39
Jar 9	9.40	19
Jar 10	9.70	49
Jar 11	9.70	42
Jar 12	9.70	44
Jar 13	10.00	50
Jar 14	10 00	50
Jar 15	10.00	48
Jar 16	10 50	50
Jar 17	10.50	50
Jar 18	10.50	50

CCF ACUTE 2

	Treatment	Morts
Jar 1	Control	2
Jar 2	Control	0
Jar 3	Control	0
Jar 4	9 00	1
Jar 5	9.00	2
Jar 6	9.00	0
Jar 7	9 40	19
Jar 8	9.40	45
Jar 9	9.40	23
Jar 10	9.70	50
Jar 11	9 70	36
Jar 12	9.70	43
Jar 13	10.00	45
Jar 14	10.00	49
Jar 15	10.00	50
Jar 16	10.50	50
Jar 17	10.50	50
Jar 18	10.50	50

BLG ACUTE

	Treatment	Morts
Jar 1	Control	0
Jar 2	Control	1
Jar 3	Control	0
Jar 4	9.00	0
Jar 5	9.00	0
Jar 6	9.00	0
Jar 7	9 40	2
Jar 8	9.40	0
Jar 9	9.40	1
Jar 10	9.70	1
Jar 11	9.70	2
Jar 12	9.70	0
Jar 13	10.00	3
Jar 14	10 00	3
Jar 15	10.00	3
Jar 16	10.50	5
Jar 17	10.50	5
Jar 18	10.50	5

FLB CHRONIC

	Treatment	Morts
Jar 1	Control	0
Jar 2	Control	2
Jar 3	Control	0
Jar 4	0	22
Jar 5	0	31
Jar 6	0	41
Jar 7	15	20
Jar 8	15	24
Jar 9	15	29
Jar 10	30	26
Jar 11	30	16
Jar 12	30	22
Jar 13	45	23
Jar 14	45	24
Jar 15	45	28
Jar 16	60	23
Jar 17	60	29
Jar 18	60	27

SMB CHRONIC

	Treatment	Morts
Jar 1	Control	0
Jar 2	Control	1
Jar 3	Control	0
Jar 4	0	41
Jar 5	0	36
Jar 6	0	37
Jar 7	15	39
Jar 8	15	35
Jar 9	15	37
Jar 10	30	25
Jar 11	30	19
Jar 12	30	27
Jar 13	45	26
Jar 14	45	24
Jar 15	45	37
Jar 16	60	20
Jar 17	60	18
Jar 18	60	32

CCF CHRONIC

	Treatment	Morts
Jar 1	Control	1
Jar 2	Control	0
Jar 3	Control	1
Jar 4	0	42
Jar 5	0	48
Jar 6	0	47
Jar 7	15	49
Jar 8	15	41
Jar 9	15	43
Jar 10	30	39
Jar 11	30	43
Jar 12	30	47
Jar 13	45	41
Jar 14	45	36
Jar 15	45	39
Jar 16	60	39
Jar 17	60	41
Jar 18	60	28

BLG CHRONIC

	Treatment	Morts
Jar 1	Control	0
Jar 2	Control	0
Jar 3	Control	0
Jar 4	0	5
Jar 5	0	5
Jar 6	0	4
Jar 7	15	2
Jar 8	15	3
Jar 9	15	5
Jar 10	30	4
Jar 11	30	4
Jar 12	30	4
Jar 13	45	4
Jar 14	45	3
Jar 15	45	3
Jar 16	60	5
Jar 17	60	4
Jar 18	60	2

APPENDIX II: LC50 values

CHEMICAL: pH **SPECIES:** Florida largemouth bass

RAW DATA:

CONCENTRATION( )	8.10	9.00	9.40	9.70	10.00	10.50
NUMBER EXPOSED:	50	50	50	50	50	50
MORTALITIES:	1	6	10	18	34	48

SPEARMAN-KARBER TRIM: 4.00%

SPEARMAN-KARBER ESTIMATES. LC50: 9.74
95% LOWER CONFIDENCE: 9.63
95% UPPER CONFIDENCE: 9.86

CHEMICAL. pH **SPECIES:** Smallmouth bass

RAW DATA:

CONCENTRATION( )	8.10	9.00	9.40	9.70	10.00	10.50
NUMBER EXPOSED:	50	50	50	50	50	50
MORTALITIES:	0	0	0	1	3	48

SPEARMAN-KARBER TRIM: 4 00%

SPEARMAN-KARBER ESTIMATES: LC50: 10.24
95% LOWER CONFIDENCE: 10.20
95% UPPER CONFIDENCE: 10.28

CHEMICAL: pH **SPECIES:** Channel catfish

RAW DATA.

CONCENTRATION( )	8.10	9.00	9 40	9 70	10.00	10.50
NUMBER EXPOSED:	50	50	50	50	50	50
MORTALITIES:	0	0	27	44	48	50

SPEARMAN-KARBER TRIM: 0.00%

SPEARMAN-KARBER ESTIMATES. LC50. 9.41
95% LOWER CONFIDENCE: 9 35
95% UPPER CONFIDENCE 9.47

CHEMICAL pH **SPECIES:** Bluegill

RAW DATA:

CONCENTRATION( )	8.10	9 00	9 40	9.70	10.00	10.50
NUMBER EXPOSED	5	5	5	5	5	5
MORTALITIES.	0	0	1	1	3	5

SPEARMAN-KARBER TRIM: 0 00%

SPEARMAN-KARBER ESTIMATES. LC50. 9.87
95% LOWER CONFIDENCE: 9 63
95% UPPER CONFIDENCE 10 12

APPENDIX III. ANOVA and Tukey HSD Multiple Comparisons

Analysis of Variance of Florida Largemouth Bass Acute

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	9757.55556	5	1951.51111	25.12316	0.00001
Error	2330.33333	30	77.67778		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.00000				
2	0.93902	1 00000			
3	0.56079	0.97316	1 00000		
4	0.03137	0.21552	0 62224	1.00000	
5	0.00001	0.00011	0.00082	0.04593	1.00000
6	0.00001	0.00001	0 00001	0 00003	0.07126

Analysis of Variance of Smallmouth Bass Acute

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	11295.47222	5	2259.09444	1299.15974	0.00001
Error	52.16667	30	1.73889		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.00000				
2	0.99992	1.00000			
3	0.99992	0.99776	1.00000		
4	0 98532	0 94945	0.99776	1.00000	
5	0 00307	0.00171	0.00547	0 01670	1.00000
6	0 00001	0.00001	0 00001	0.00001	0.00001

Analysis of Variance of Channel Catfish Acute

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	16011.25000	5	3202.25000	119.85964	0.00001
Error	801.50000	30	26.71667		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.00000				
2	1.00000	1.00000			
3	0.00001	0.00001	1.00000		
4	0.00001	0 00001	0.00009	1.00000	
5	0.00001	0.00001	0.00001	0.62747	1.00000
6	0.00001	0.00001	0.00001	0.36024	0.99753

Analysis of Variance of Bluegill Acute

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	54.94444	5	10.98889	28.25714	0.00001
Error	4.66667	12	0.38889		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.00000				
2	0.98384	1.00000			
3	0.77512	0.41342	1.00000		
4	0.77512	0.41342	1.00000	1.00000	
5	0.00222	0.00081	0.01915	0.01915	1.00000
6	0.00002	0.00001	0.00006	0.00006	0.01915

Analysis of Variance of Florida Largemouth Bass Chronic

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	642.66667	4	160.66667	1 31838	0.32801
Error	1218 66667	10	121.86667		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1 00000				
2	0.55477	1.00000			
3	0 24798	0.95953	1.00000		
4	0.63814	0.99987	0.92053	1.00000	
5	0.79831	0.99071	0.79831	0 99803	1.00000

Analysis of Variance of Smallmouth Bass Chronic

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	2380.26667	4	595.06667	5.52351	0.01305
Error	1077.33333	10	107.73333		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.00000				
2	0.99919	1 00000			
3	0.04338	0.06256	1.00000		
4	0.28200	0.38154	0.72011	1.00000	
5	0.03839	0.05538	0.99999	0.67648	1.00000

Analysis of Variance of Channel Catfish Chronic

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	790.93333	4	197.73333	2.49663	0.10969
Error	792.00000	10	79.20000		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.00000				
2	0.99548	1.00000			
3	0.94346	0.99548	1.00000		
4	0.36376	0.55116	0.75559	1.00000	
5	0.13128	0.22356	0.36376	0.94346	1.00000

Analysis of Variance of Bluegill Chronic

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	1493.33333	4	373.33333	0.87500	0.51211
Error	4266.66667	10	426 66667		

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.00000				
2	0.53928	1.00000			
3	0.92763	0.92763	1.00000		
4	0.53928	1.00000	0.92763	1.00000	
5	0.75920	0 99400	0.99400	0.99400	1.00000

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VITA

Nathan Pence was born in Garland, Texas, on April 1, 1976, the son of Linda and Bobby Pence. After graduating from high school with honors in 1994, he was accepted into Southwest Texas State University in San Marcos, Texas. In 1998, he received his Bachelor of Science degree from Southwest Texas State University in Aquatic Biology. During the following years Nathan was employed by Texas Parks and Wildlife at A.E. Wood Fish Hatchery in San Marcos. In August of 2000, he entered the Graduate College of Southwest Texas State University, San Marcos, Texas, and he continued to work for Texas Parks and Wildlife full-time.

Permanent address: 908 Allen

San Marcos, Texas 78666